# ADDENDUM

We recently showed (Bonazzi *et al.* 7, 570–580; 2005) that the protein CtBP3/BARS (BARS) controls basolateral but not apical traffic from the Golgi to the plasma membrane. One of the several lines of evidence supporting this conclusion was that a 'smart pool' of siRNA duplexes (Dharmacon, Lafayette, CA) against the human sequence of BARS caused the selective knockdown of BARS and the inhibition of basolateral but not of apical traffic in COS-7 cells (Fig. 3e; Bonazzi *et al.*; 2005). Because the BARS nucleotide sequence of COS-7 cells (of monkey origin) is unknown, we have now: (1) obtained the COS-7 BARS sequence (V. Hsu, personal communication); and (2) performed rescue-of-function experiments in siRNA-treated COS-7 cells by restoring their BARS cytosolic levels with recombinant rat protein.

We report that: (1) COS-7 BARS contains a sequence (CCGTCAAGCAGATGAGACA) that perfectly matches one of the siRNA oligonucleotides in the Dharmacon pool, thus justifying the published effects of this pool in COS-7 cells; and (2) COS-7 cells that were depleted of BARS by siRNA and then were treated exactly as described in the experiment in Fig. 3e (Bonazzi *et al.*; 2005) regain normal levels of basolateral transport to the plasma membrane upon microinjection with recombinant BARS (Table 1). These results confirm the specificity of the siRNA effects on basolateral traffic shown in Fig. 3e (Bonazzi *et al.*; 2005).

**Table 1** Specificity of the effects of siRNAs against BARS on VSVG traffic

| | | | | |
|---|---|---|---|---|
| VSVG at the plasma membrane (arbitrary units) | 100 ± 9 | 5 ± 1 | 5 ± 1 | 78 ± 8 |
| siRNA | NT | + | + | + |
| Injection | – | – | GST | GST–BARS |

COS-7 cells were co-transfected with the anti-BARS siRNAs (+), or with non-targeting siRNAs (NT), and VSVG–GFP. Thirty hours later, they were incubated for 16 h at 40 °C and then at 20 °C for 2 h to accumulate VSVG in the *trans*-Golgi network. During this 20 °C block, the cells were injected with recombinant GST–BARS (0.2–2 mg $ml^{-1}$ in the pipette) or GST. Subsequently, they were shifted to 32 °C to release the traffic block, and 40 min later they were fixed and analysed for their levels of VSVG at the plasma membrane as described (Bonazzi *et al.*; 2005). Values are means ± s.d. of 15 measurements per experimental condition in each of three independent experiments, and are expressed as a percentage of VSVG arrival at the plasma membrane in control cells.